

Effects of nicotine and cigarette smoking on the human lymphocyte transcriptome

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ABSTRACT

Nicotine has a wide range of toxic effects on neuronal and non-neuronal cells, including those on addiction and carcinogenic pathways through nicotinic receptors and other not well-defined pathways. Using Affymetrix HG-U133A GeneChips, we investigated global gene expression in peripheral lymphocytes from 22 current healthy smokers. Isolated lymphocytes from blood, PHA-stimulated cultured lymphocytes and PHA-stimulated lymphocytes co-cultured with nicotine were assayed. Total RNA was isolated using TRIzol reagent and purified with RNeasy kit. All other procedures were performed according to manufacture's protocols. The array data were analyzed with dChip V1.3 software based on both statistical significance levels and fold-change differences. Arrays that failed to pass several quality-control checkpoints were excluded. For cultured lymphocytes *in vitro*, expression patterns of some 1789 genes were significantly altered after exposure to nicotine for 4 hours. But only 99 were nicotine-associated genes reported in the literature. A small subset of approximately 74 genes had an absolute fold-change >1.5. Because nicotine is the major component of cigarette smoking, we examined the transcriptomes of uncultured lymphocytes. Compared with light smokers, the heavy smokers who smoked 20 cigarettes or more per day gave a different expression signature. A sum of 845 genes were changed. Among them, 82 overlapped with the above 1789 nicotine-associated genes and some were immune genes and oncogenes. About 226 had an absolute fold-change >1.5, but only 9 of which also appeared in the list of 74 genes after nicotine treatment, few of which were nicotine-related. These genes included phospholipase A2 receptor 1, PLC beta 1, MCF2L2. The data from this study demonstrate that nicotine treatment *in vitro* changes a large set of gene expression pathways and more exposure to cigarette smoking *in vivo* also causes a different gene expression signature. Those genes that are nicotine responsive both in *in vitro* and *in vivo* deserve further attention as potentially critically important in addition or carcinogenic pathways, or both.

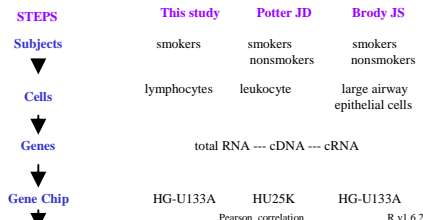
INTRODUCTION

Nicotine is a major component of cigarette smoking. Currently it is also used for replacement therapy on smoking cessation and PREPS.

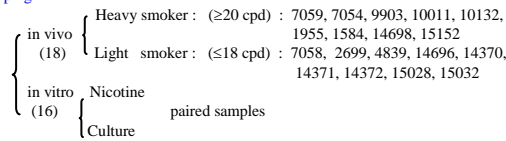
Nicotine has a wide range of toxic effects. It is regarded as the major addictive agent. Nicotine is also a survival agonist. Nicotine signaling can occur as a result of activation of either the classical (nAChR) and/or non-classical (G protein-coupled) nicotine receptor pathways in non-neuronal cells. However, human evidence for this is lacking.

In this study, we examined the gene expression signature of human lymphocytes exposed to nicotine or cigarette smoke to identify some functional biological markers.

MATERIALS AND METHODS



Grouping



RESULTS

1. Gene expression patterns after exposure to nicotine

Tab 1. Numbers of altered genes after nicotine treatment

Groups	Fold change	P value	Fold + P
N / C	74	1789	18
H / L	226	845	41
overlap	9	83	1(inversin)

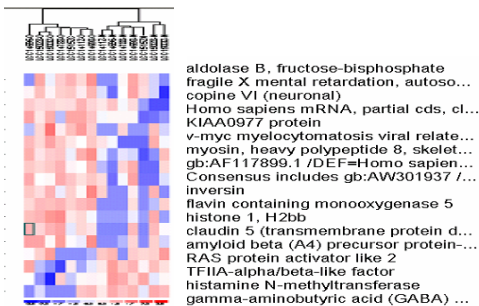


Fig 1. Hierarchical clustering of cultured samples according to the expression of 18 most differentially expressed genes between the nicotine-treated and cultured alone lymphocytes.

Tab. 2. Pathways and genes affected by nicotine treatment

Pathway	Representative Genes	Changes
Signal transduction	G protein-coupled receptor 51 TNFRSF1A-associated via death domain Dual specificity phosphatase 1 MAPK 12 Calcium channel, alpha 1A subunit	Up-regulated
Oncogenesis	Cdk inhibitor 1A(p21, Cip1) RAS-related on chromosome 22 RAS2, member RAS oncogene family-like RAS14, member RAS oncogene family GABA A receptor, alpha 6 Thyroid stimulating hormone receptor	Up-regulated
Defense/immunity protein activity	NK cell receptor 2B4 BCG-induced gene in monocytes, clone 103 Aquaporin 5 Dendritic cell protein	Up-regulated
Cell motility	FGF receptor 2 Integrin binding sialoprotein Myosin, light polypeptide 5, regulatory Axonal transport of synaptic vesicles	Up-regulated
Metabolism	Histamine N-methyltransferase Dopamine receptor D5 Aldolase B, fructose-bisphosphate Ubiquitin 3	Up-regulated
RNA binding and metabolism	Histone 1, H2bb NADH dehydrogenase flavoprotein 2, 24kDa Claudin 5	Down-regulated
Porter activity	Solute carrier family 6 Forkhead box A2	Down-regulated

2. Genes differentially expressed between heavy and light smokers

probe set	gene	fold change	P value
210417_s	phosphatidylinositol 4-kinase, catalytic, beta polypeptide	4.72	0.033134
213197_at	astrotactin	4.05	0.019147
206249_at	mitogen-activated protein kinase kinase kinase	3.38	0.010297
221212_x	polybromo 1	2.36	0.027047
219753_at	stromal antigen 3	2.13	0.034926
210432_s	sodium channel, voltage-gated, type III, alpha polypeptide	2.09	0.031313
206459_s	wingless-type MMTV integration site family, member 2	2	0.016462
215312_at	Human DNA damage repair and recombination	1.93	0.008992
222084_s	SET binding factor 1	1.93	0.035923
213674_x	immunoglobulin heavy constant gamma 3 (G3m)	1.82	0.020544
212865_s	collagen, type XIV, alpha 1 (undulin)	1.81	0.026783
215546_at	proteasome (prosome, macropain) 26S subunit	1.81	0.017889
216227_at	HCGII-7 protein	1.8	0.042728
221117_at	ncaml	1.79	0.021129
221239_s	SH2 domain containing phosphatase anchor protein 1	1.76	0.016604
211925_s	phospholipase A2 receptor 1, 180kDa	1.64	0.033642
204233_s	choline kinase	1.63	0.00965
202859_x	interleukin 8	-1.6	0.032496
202007_at	nidogen (enactin)	-1.65	0.025765
205403_at	interleukin 1 receptor, type II	-1.68	0.022021
206120_at	CD33 antigen (gp67)	-1.78	0.027551
211054_at	inversin	-2.84	0.026074
201044_x	dual specificity phosphatase 1	-3.37	0.047136

3. Genes that are nicotine responsive both in *in vitro* and *in vivo*

probe set	gene	change
215112_x_at	MCF 2 cell line derived transforming sequence-like 2	up-regulated
210194_at	phospholipase A2 receptor 1, 180kDa	
216227_at	HCGII-7 protein	
209991_x_at	G protein-coupled receptor 51	
203849_s	axonal transport of synaptic vesicles	
213197_at	astrotactin	
220192_x	prostate epithelium-specific Ets transcription factor	
210718_s	NEB2	
214894_x	microtubule-actin crosslinking factor 1	
201294_s	SOCS box-containing WD protein SWIP-1	
216968_at	marfan-binding lectin serine protease 2	
220118_at	testis zinc finger protein	
200927_s	RAB14, member RAS oncogene family	
209592_s	WD-repeat protein	
221002_at	tetraspanin similar to TM4SF9	
209995_s	T-cell leukemia/lymphoma 1A	
214107_x	aminopeptidase puromycin sensitive	
200731_s	protein tyrosine phosphatase type IVA, member 1	down-regulated
214732_at	Sp1 transcription factor	
221724_s	C-type lectin, superfamily member 6	
203892_at	VAP four-disulfide core domain 2	
210808_s	NADPH oxidase 1	
202000_at	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 6, 14kDa	
205141_at	angiotensin, ribonuclease, RNase A family, 5	
209686_at	S100 calcium binding protein, beta (neuronal)	
204397_at	ectoderm microtubule associated protein like 2	
207918_s	testis specific protein, Y-linked	
211054_at	inversin	

SUMMARY AND CONCLUSIONS

- Nicotine treatment *in vitro* changes a large set of gene expression pathways
- It is possible to distinguish between 18 heavy or light smokers on the basis of mRNA expression in peripheral lymphocytes; More exposure to cigarette smoking *in vivo* causes a biologically different gene expression signature
- Those genes that are nicotine responsive both in *in vitro* and *in vivo* deserve further attention as potentially critically important in addition or carcinogenic pathways, or both

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